

(FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001

L1 60112 S PEG
L2 0 S POLYETHYLENE ADJ GLYCOL
L3 110582 S POLYETHYLENE (A) GLYCOL
L4 146676 S L1 OR L3
L5 885718 S MOLECULAR(W) WEIGHT
L6 40836 S "8000" OR "10000" OR "18000"
L7 5333 S L5 AND L6
L8 576 S L4 AND L7
L9 124212 S COVALENT OR IMMOBILI?
L10 247404 S (SUPEROXIDE (A)DISMUTASE?) OR CATALASE? OR (GLUTATHIONE(A)
PE
L11 1277 S L9 AND L10
L12 0 S L8 AND L11
L13 113 S L11 AND L4
L14 30 S L5 AND L13
L15 16 DUP REM L14 (14 DUPLICATES REMOVED)
L16 2362944 S WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?
L17 0 S L15 AND L16
L18 70993 S ISOCYANATE?
L19 0 S L18 AND L15
L20 48865 S DIISOCYANATE?
L21 0 S L15 AND L20
L22 0 S L15 AND (UREA OR URETHANE?)
L23 6 S L15 AND AMINO
L24 6 DUP REM L23 (0 DUPLICATES REMOVED)
E ETTNER N/AU
L25 26 S E3
L26 0 S L25 AND L3
L27 6 S L24 AND L4
L28 6 DUP REM L27 (0 DUPLICATES REMOVED)
L29 0 S L28 AND L6
E SCHINK M/AU
L30 34 S E3
L31 0 S L4 AND L30
E SCHREIBER J/AU
L32 811 S E3
L33 0 S L32 AND L4
E MEIER W/AU
L34 1206 S E3
L35 3 S L4 AND L34
L36 2 DUP REM L35 (1 DUPLICATE REMOVED)
E SAUER M/AU
L37 550 S E3
L38 0 S L4 AND L37

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NEWS 3 Feb 06 Engineering Information Encompass files have new names
NEWS 4 Feb 16 TOXLINE no longer being updated
NEWS 5 Apr 23 Search Derwent WPINDEX by chemical structure
NEWS 6 Apr 23 PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA
NEWS 7 May 07 DGENE Reload
NEWS 8 Jun 20 Published patent applications (A1) are now in USPATFULL
NEWS 9 JUL 13 New SDI alert frequency now available in Derwent's
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NEWS 13 Sep 17 IMSworld Pharmaceutical Company Directory name change
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NEWS 14 Oct 09 Korean abstracts now included in Derwent World Patents
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NEWS 15 Oct 09 Number of Derwent World Patents Index updates increased
NEWS 16 Oct 15 Calculated properties now in the REGISTRY/ZREGISTRY File
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NEWS 18 Oct 22 DGENE GETSIM has been improved
NEWS 19 Oct 29 AAASD no longer available

NEWS EXPRESS August 15 CURRENT WINDOWS VERSION IS V6.0c,
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AND CURRENT DISCOVER FILE IS DATED 07 AUGUST 2001
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FILE 'LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001
COPYRIGHT (C) 2001 Cambridge Scientific Abstracts (CSA)

=> s peg

L1 60112 PEG

=> s polyethylene adj glycol

L2 0 POLYETHYLENE ADJ GLYCOL

=> s polyethylene (a) glycol

L3 110582 POLYETHYLENE (A) GLYCOL

=> s l1 or l3

L4 146676 L1 OR L3

=> s molecular(w) weight

L5 885718 MOLECULAR(W) WEIGHT

=> s "8000" or "10000" or "18000"

L6 40836 "8000" OR "10000" OR "18000"

=> s l5 and l6

L7 5333 L5 AND L6

=> s l4 and l7

L8 576 L4 AND L7

=> s covalent or immobili?

L9 124212 COVALENT OR IMMOBILI?

=> s (superoxide (a)dismutase?) or catalase? or (glutathione(a) peroxidase?)
or myeloperoxidase?

7 FILES SEARCHED...

L10 247404 (SUPEROXIDE (A) DISMUTASE?) OR CATALASE? OR (GLUTATHIONE (A) PEROXIDASE?) OR MYELOPEROXIDASE?

=> s l9 and l10

L11 1277 L9 AND L10

=> s l8 and l11

L12 0 L8 AND L11

=> s l11 and l4

L13 113 L11 AND L4

=> s l5 and l13

L14 30 L5 AND L13

=> dup rem l14

PROCESSING COMPLETED FOR L14

L15 16.DUP REM L14 (14 DUPLICATES REMOVED)

=> s wound or bandage or compress? or plaster? or sheet? or film?

L16 2362944 WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?

=> s l15 and l16

L17 0 L15 AND L16

=> d l15 1-16 ibib ab

L15 ANSWER 1 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)

ACCESSION NUMBER: 2001:114920 SCISEARCH

THE GENUINE ARTICLE: 397WZ

TITLE: Peptide and protein PEGylation: a review of problems and solutions

AUTHOR: Veronese F M (Reprint)

CORPORATE SOURCE: Univ Padua, Ctr Chem Invest Drugs, CNR, Dept Pharmaceut Sci, Via F Marzolo 5, I-35131 Padua, Italy (Reprint);

Univ

Padua, Ctr Chem Invest Drugs, CNR, Dept Pharmaceut Sci, I-35131 Padua, Italy

COUNTRY OF AUTHOR: Italy

SOURCE: BIOMATERIALS, (MAR 2001) Vol. 22, No. 5, pp. 405-417.

Publisher: ELSEVIER SCI LTD, THE BOULEVARD, LANGFORD

LANE,

KIDLINGTON, OXFORD OX5 1GB, OXON, ENGLAND.

ISSN: 0142-9612.

DOCUMENT TYPE: General Review; Journal

LANGUAGE: English

REFERENCE COUNT: 83

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The paper discusses general problems in using PEG for conjugation to high or low **molecular weight** molecules. Methods of binding PEG to different functional groups in macromolecules is reported together with their eventual limitations. Problems encountered in conjugation. such as the evaluation of the number of PEG chains bound, the localisation of the site of conjugation in polypeptides and the procedure to direct PEGylation to the desired site in the molecule are discussed. Finally, the paper reports on more specific

L15 ANSWER 2 OF 16 MEDLINE DUPLICATE 1
 ACCESSION NUMBER: 1999440547 MEDLINE
 DOCUMENT NUMBER: 99440547 PubMed ID: 10510847
 TITLE: Bioconjugation in pharmaceutical chemistry.
 AUTHOR: Veronese F M; Morpurgo M
 CORPORATE SOURCE: Department of Pharmaceutical Sciences, University of Padua,
 Italy.. veronese@pdfar3.dsfarm.unipd.it
 SOURCE: FARMACO, (1999 Aug 30) 54 (8) 497-516. Ref: 149
 Journal code: ACZ; 8912641. ISSN: 0014-827X.
 PUB. COUNTRY: Italy
 Journal; Article; (JOURNAL ARTICLE)
 General Review; (REVIEW)
 (REVIEW, TUTORIAL)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199910
 ENTRY DATE: Entered STN: 20000111
 Last Updated on STN: 20000111
 Entered Medline: 19991025

AB Polymer conjugation is of increasing interest in pharmaceutical chemistry for delivering drugs of simple structure or complex compounds such peptides, enzymes and oligonucleotides. For long time drugs, mainly with antitumoral activity, have been coupled to natural or synthetic polymers with the purpose of increasing their blood permanence time, taking advantage of the increased mass that reduces kidney ultrafiltration. However only recently complex constructs were devised that exploit the 'enhanced permeability and retention' (EPR) effect for an efficient tumor targeting, the high **molecular weight** for adsorption or receptor mediated endocytosis and finally a lysosomotropic targeting, taking advantage of acid labile bonds or cathepsin susceptible

polypeptide

spacers between polymer and drug. New original, very active conjugates of this type, as those based on poly(hydroxyacrylate) polymers, are already in advanced state of development. Labile oligonucleotides, including antisense drugs, were also successfully coupled to polymers in view of an increased cell penetration and stabilization towards nucleases. However, the most active research activity resides in the field of polypeptides

and

proteins delivery, mainly for the two following reasons: first of all because a great number of therapeutically interesting compounds are now being produced by genetic engineering in large quantity and, secondly, because these products are difficult to administer to patients for

several

inherent drawbacks. Proteins are in fact easily digested by many endo-

and

exo-peptidases present in blood or in other body districts; most of them are immunogenic to some extent and, finally, they are rapidly excreted by kidney ultrafiltration. **Covalent** polymer conjugation at protein surface was demonstrated to reduce or eliminate these problems, since the bound polymer behaves like a shield hindering the approach of proteolytic enzymes, antibodies, or antigen processing cell. Furthermore, the

increase

of the **molecular weight** of the conjugate allows to overcome the kidney elimination threshold. Many successful results were already obtained in peptides and proteins, conjugated mainly to water soluble or amphiphilic polymers like poly(ethylene glycol) (**PEG**), dextrans, or styrenemaleic acid anhydride. Among the most successful are the conjugates of asparaginase, interleukin-2 or -6 and neocarzinostatin, to remind some antitumor agents, adenosine deaminase employed in a genetic disease treatment, **superoxide**

dismutase as scavenger of toxic radicals, hemoglobin as oxygen carrier and urokinase and streptokinase as proteins with antithrombotic activity. In pharmaceutical chemistry the conjugation with polymers is also of great importance for synthetic applications since many enzymes without loss of catalytic activity become soluble in organic solvents where many drug precursors are. The various and often difficult chemical problems encountered in conjugation of so many different products

prompted

the development of many synthetic procedures, all characterized by high specificity and mild condition of reaction, now known as 'bioconjugation chemistry'. Bioconjugation developed also the design of new tailor-made polymers with the wanted **molecular weight**, shape,

structure and with the functional groups needed for coupling at the wanted positions in the chain.

L15 ANSWER 3 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)

ACCESSION NUMBER: 97:286673 SCISEARCH

THE GENUINE ARTICLE: WR444

TITLE: Prolongation of the serum half-life period of **superoxide dismutase** by poly(ethylene glycol) modification

AUTHOR: Nakaoka R; Tabata Y; Yamaoka T; Ikada Y (Reprint)

CORPORATE SOURCE: KYOTO UNIV, BIOMED ENGN RES CTR, SAKYO KU, 53 KAWAHARA CHO, KYOTO 60601, JAPAN (Reprint); KYOTO UNIV, BIOMED

ENGN

RES CTR, SAKYO KU, KYOTO 60601, JAPAN

COUNTRY OF AUTHOR: JAPAN

SOURCE: JOURNAL OF CONTROLLED RELEASE, (2 JUN 1997) Vol. 46, No. 3, pp. 253-261.

Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.

ISSN: 0168-3659.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 33

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Superoxide dismutase** (SOD) was chemically modified using poly(ethylene glycol) (**PEG**) with different molecular weights to prepare **PEG**-SOD conjugates with different extents of modification. The body distribution of the conjugates intravenously injected to mice was investigated to assess the influence of modification on the serum half-life period of SOD. The SOD modification with **PEG** was effective in lowering the elimination rate of SOD from the blood circulation without any change in the distribution pattern of organs

other than the kidney. The **molecular weight** of **PEG** used for modification and the modification extent have a minimum effect on the half-life of the SOD. The half-life of the SOD and its **PEG** conjugates have a similar dependency on the apparent **molecular weight** as the **PEG** molecules. This indicates that the half-life of SOD and the **PEG** conjugates are mainly determined by their molecular size.

L15 ANSWER 4 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)

ACCESSION NUMBER: 1998:12669 SCISEARCH

THE GENUINE ARTICLE: BK03M

TITLE: Conjugation of high-**molecular weight** poly(ethylene glycol) to cytokines:

Granulocyte-macrophage

colony-stimulating factors as model substrates

AUTHOR: Sherman M R (Reprint); Williams L D; Saifer M G P; French J A; Kwak L W; Oppenheim J J

CORPORATE SOURCE: MT VIEW PHARMACEUT INC, 871-L IND PK, MENLO PK, CA 94025 (Reprint); NCI, FREDERICK CANC RES & DEV CTR, FREDERICK,

COUNTRY OF AUTHOR: ACS SYMPOSIUM SERIES, (FEB 1997) Vol. 680, pp. 155-169.
 SOURCE: Publisher: AMER CHEMICAL SOC, 1155 SIXTEENTH ST NW,
 WASHINGTON, DC 20036.
 ISSN: 0097-6156.
 DOCUMENT TYPE: General Review; Journal
 LANGUAGE: English
 REFERENCE COUNT: 71

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The ability of the small receptor-binding protein, recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF), to increase the abundance of certain blood cell types in mice was enhanced markedly by covalent attachment of a single long strand of PEG (30-40 kDa). Potency was not increased further by coupling a second strand. Such conjugates can be synthesized efficiently by reaction of protein amino groups with PEG propionaldehydes in the presence of NaBH₃CN or with PEG p-nitrophenyl carbonates. Both methods have been used to prepare recombinant human GM-CSF conjugates of predetermined composition, e.g. PEG(1)GM-CSF and PEG(2)GM-CSF, in high yield. These compounds, or analogous derivatives of other cytokines, purified by ion-exchange and size-exclusion chromatography, may be suitable candidates for pharmaceutical development.

L15 ANSWER 5 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
 ACCESSION NUMBER: 97:222875 SCISEARCH
 THE GENUINE ARTICLE: WN017
 TITLE: A simple and efficient method for preparation of monomethoxypolyethylene glycol activated with p-nitrophenylchloroformate and its application to modification of L-asparaginase
 AUTHOR: Kito M; Miron T; Wilchek M; Kojima N; Ohishi N; Yagi K (Reprint)
 CORPORATE SOURCE: INST APPL BIOCHEM, YAGI MEM PK, GIFU 50501, JAPAN (Reprint); INST APPL BIOCHEM, GIFU 50501, JAPAN; WEIZMANN INST SCI, DEPT BIOPHYS, IL-76100 REHOVOT, ISRAEL
 COUNTRY OF AUTHOR: JAPAN; ISRAEL
 SOURCE: JOURNAL OF CLINICAL BIOCHEMISTRY AND NUTRITION, (SEP 1996)
 Vol. 21, No. 2, pp. 101-111.
 Publisher: INST APPLIED BIOCHEMISTRY, YAGI MEMORIAL PARK, MITAKE GIFU 505-01, JAPAN.
 ISSN: 0912-0009.
 DOCUMENT TYPE: Article; Journal
 FILE SEGMENT: LIFE
 LANGUAGE: English
 REFERENCE COUNT: 28

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An improved, simple and efficient method for preparation of monomethoxypolyethylene glycol (PEG) activated with p-nitrophenylchloroformate (PNP-PEG) and its use as a potent modifier of protein under mild conditions are described. Modification of bovine serum albumin with PNP-PEG was compared with that done with PEG activated with N,N'-carbonyldiimidazole or cyanuric chloride. The reaction of PEG, activated with either p-nitrophenylchloroformate or cyanuric chloride, with bovine serum albumin at 4 degrees C reached a plateau within 1 h, whereas protein modification using PEG activated with N,N'-carbonyldiimidazole was rather slow and gave a low yield. The remaining activity of L-asparaginase modified with PNP-PEG was much higher than that of the enzyme modified to the same degree with PEG activated with cyanuric chloride. At a 20 molar excess of PNP-PEG having a molecular weight of 5,000, 55% of the free amino acid

groups were modified at 4 degrees C for 2 h, and the modified enzyme still had 33% residual enzyme activity. Immunochemical studies showed that the highly modified enzyme (67% modification with 18% residual enzyme activity) had lost its immunogenicity and had become much less sensitive to protease digestion.

L15 ANSWER 6 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 96:257769 SCISEARCH
THE GENUINE ARTICLE: UB814
TITLE: SYNTHESIS, CHARACTERIZATION AND PROPERTIES OF SIALYLATED
CATALASE
AUTHOR: FERNANDES A I; GREGORIADIS G (Reprint)
CORPORATE SOURCE: UNIV LONDON, SCH PHARM, CTR DRUG DELIVERY RES, 29-39
BRUNSWICK SQ, LONDON WC1N 1AX, ENGLAND (Reprint); UNIV
LONDON, SCH PHARM, CTR DRUG DELIVERY RES, LONDON WC1N
1AX,
ENGLAND
COUNTRY OF AUTHOR: ENGLAND
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA-PROTEIN STRUCTURE AND
MOLECULAR ENZYMOLOGY, (07 MAR 1996) Vol. 1293, No. 1, pp.
90-96.
ISSN: 0167-4838.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 35

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Colominic acid (CA), a alpha-(2 --> 8) N-acetylneuraminic acid (sialic acid) polymer (average molecular weight of 10 kDa) was activated by periodate oxidation of carbon 7 at the non-reducing end of the saccharide. The oxidized CA was then coupled to **catalase** by reductive amination in the presence of sodium cyanoborohydride. The extent of sialylation of **catalase**, estimated by ammonium sulfate precipitation as 3.8 +/- 0.4 (mean +/- S.D.) moles of CA per mole of **catalase**, did not improve significantly when depolymerized CA was used in the coupling reaction. At the end of the coupling reaction, sialylated **catalase** exhibited a two-fold (70%) retention of initial activity compared to enzyme controls (29-35%) subjected to the same conditions. Formation of sialylated **catalase** was confirmed by ammonium sulfate or trichloroacetic acid precipitation, molecular sieve chromatography and SDS-PAGE electrophoresis. Enzyme kinetics studies revealed an increase in the apparent K-m of the enzyme from 70.0 (native) to 122.9 mmol l(-1) H2O2 (sialylated **catalase**) indicating a reduction of enzyme affinity for the substrate (hydrogen peroxide) on sialylation. Compared to native enzyme, sialylated **catalase** was much more stable in the presence of specific proteinases, completely resisting degradation by chymotrypsin and losing only some of its activity in the presence of trypsin. The increased stability conferred to **catalase** by sialylation agrees with similar observations on enzymes modified by other hydrophilic molecules (e.g., monomethoxypoly(ethyleneglycol)) and suggests that steric stabilization with the biodegradable polysialic acid may prove an alternative means to render therapeutic proteins more effective in vivo.

L15 ANSWER 7 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 95:443915 SCISEARCH
THE GENUINE ARTICLE: RE939
TITLE: COMPARISON OF BODY DISTRIBUTION OF POLY(VINYL ALCOHOL)
WITH OTHER WATER-SOLUBLE POLYMERS AFTER INTRAVENOUS
ADMINISTRATION
AUTHOR: YAMAOKA T; TABATA Y; IKADA Y (Reprint)
CORPORATE SOURCE: KYOTO UNIV, BIOMED ENGN RES CTR, SAKYO KU, 53 KAWAHARA

COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF PHARMACY AND PHARMACOLOGY, (JUN 1995) Vol. 47,
No. 6, pp. 479-486.
ISSN: 0022-3573.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The body distribution of poly(vinyl alcohol) (PVA) with molecular weights (MW) from 14800 to 434000 Da was investigated after intravenous administration and compared with that of other water-soluble polymers such as poly(ethylene glycol) (PEG), gelatin, dextran, and pullulan. The half-life of PVA in the circulation was prolonged from 90 min (MW 14800Da) to 23 h (MW 434000 Da), similar to that of PEG which had a half-life of 30 min (MW 6000) and 20 h (MW 170000). However, the half-life of PVA was much longer than that of other polymers when compared at a similar molecular weight. PVA was located in most organs but with very small accumulation. An insignificant interaction of PVA with cell components, such as macrophages and blood cells, was observed. Similar to PEG, the excretion rate of PVA at the glomeruli was rapidly reduced around 30000 Da, as the molecular weight increased. These results indicate that the half-life of intravenously injected PVA in the blood was mainly determined by the permeation characteristics of the kidney.

L15 ANSWER 8 OF 16 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:213658 HCAPLUS
DOCUMENT NUMBER: 124:279129
TITLE: The effect of human recombinant **superoxide dismutase** conjugated with **polyethylene glycol** on the hepatic toxicity of acetaminophen
AUTHOR(S): Yong, Chul Soon; Park, Kyong-Ah; Oh, Doo-Man
CORPORATE SOURCE: Coll. Pharmacy, Yeungnam Univ., Gyongsan, 712-749, S. Korea
SOURCE: Yakche Hakhoechi (1995), 25(4), 313-22
CODEN: YAHAEX; ISSN: 0259-2347
DOCUMENT TYPE: Journal
LANGUAGE: Korean

AB The **covalent** conjugation of human recombinant **superoxide dismutase** (hrSOD) with trichloro-s-triazine-activated **polyethylene glycol** (PGE) 5000 formed sol. conjugates with mol. wt. of 92 kD, which retained 90.apprx.98% of original activity with a markedly prolonged plasma half-life of enzyme activity. The effect of hrSOD-Peg conjugates on acetaminophen (ACP)-induced hepatotoxicity was tested in male rats which were pretreated with 3-methylcholanthrene. HrSOD-PEG conjugates inhibited the hepatotoxicity produced by ACP; on the other hand, native hrSOD had no protective effect. The above results indicated that oxygen radicals might participate in the mechanism of the ACP-induced hepatotoxicity and that polymer conjugated-protein drugs with prolonged half-lives could be employed as an effective therapeutic agent.

L15 ANSWER 9 OF 16 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 2

ACCESSION NUMBER: 95309405 EMBASE
DOCUMENT NUMBER: 1995309405
TITLE: Chemistry of **polyethylene glycol**

conjugates with biologically.
AUTHOR: Zolubsky S.
CORPORATE SOURCE: SEQUUS Pharmaceuticals Inc, 960 Hamilton Court, Menlo Park, CA 94025, United States
SOURCE: Advanced Drug Delivery Reviews, (1995) 16/2-3 (157-182).
ISSN: 0169-409X CODEN: ADDREP
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Polyethylene glycol (PEG) is widely used as a covalent modifier of biological macromolecules and particulates as well as a carrier for low molecular weight drugs. In the first two instances proteins and liposomes are of particular importance. Their conjugates with PEG often possess the ability to avoid quick recognition and clearance in vivo, that their unconjugated counterparts are suffering from. In this review (with 133 references) methods for preparation of PEG conjugates with various biologically active compounds are summarized. Since the bulk of the published work in this field involves proteins, drugs, and lipids, an appropriate emphasis is given to the conjugates of these compounds. While the first two types of PEG conjugates are usually intended for a direct use as therapeutics, PEG-lipids are mainly utilized for formation of long-circulating liposomes. Particular attention is paid to the comparative attributes of various reactive PEG derivatives, properties of the linkages formed, and possible side reactions. The relationships between various conjugation strategies and their influence on the relevant biological properties and/or on in vivo performance of the corresponding conjugates is also discussed.

L15 ANSWER 10 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 94:220960 SCISEARCH
THE GENUINE ARTICLE: NF270
TITLE: DISTRIBUTION AND TISSUE UPTAKE OF POLY(ETHYLENE GLYCOL) WITH DIFFERENT MOLECULAR-WEIGHTS AFTER INTRAVENOUS ADMINISTRATION TO MICE
AUTHOR: YAMAOKA T; TABATA Y; IKADA Y (Reprint)
CORPORATE SOURCE: KYOTO UNIV, BIOMED ENGN RES CTR, SAKYO KU, 53 KAWAHARA CHO
SHOGAIN, KYOTO 606, JAPAN (Reprint); KYOTO UNIV, BIOMED ENGN RES CTR, SAKYO KU, KYOTO 606, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF PHARMACEUTICAL SCIENCES, (APR 1994) Vol. 83, No. 4, pp. 601-606.
ISSN: 0022-3549.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 38

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB After intravenous (iv) injection of I-125-labeled poly(ethylene glycol) (PEG) with different molecular weights to mice, the radioactivity of the organs was measured to pharmacokinetically analyze the body distribution of PEG according to a two-compartment model. High molecular weight PEGs were retained in the blood circulation for a longer period than low molecular weight PEGs. The terminal half-life of PEG in the circulation extended from 18 min to 1 day as the PEG

molecular weight increased from 6000 to 190 000. PEG tended to accumulate in the tissues/organs such as muscle, skin, bone, and the liver to a higher extent than the other organs, irrespective of the molecular weight. The time dependence of tissue accumulation was based on the vascular permeability. The results of pharmacokinetic analysis suggested that small PEG tended to freely translocate from the circulation to extravascular tissues

and to return to the blood circulation again by diffusion, whereas large PEG translocated more slowly to extravascular tissues. Urinary clearance decreased with increasing PEG molecular weight, similar to the tissue clearance, whereas liver clearance increased with the increasing PEG molecular weight, after passing a minimum around the molecular weight of 50 000. PEG uptake by Kupffer cells was enhanced as the molecular weight became >50 000.

L15 ANSWER 11 OF 16 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 3
ACCESSION NUMBER: 1995:16065 BIOSIS
DOCUMENT NUMBER: PREV199598030365
TITLE: Acylation of amino functions of proteins with monomethoxypoly (ethylene glycol)-N-succinimide carbonate.
AUTHOR(S): Nijs, Michelle; Gelbcke, Michel; Azarkan, Mohamed; Brygier, Jeanne; Guermant, Claude; Baeyens-Volant, Danielle; Musu, Tony; Paul, Claudine; Looze, Yvan (1)
CORPORATE SOURCE: (1) Protein Chem. Unit, Fac. Med., Univ. Brussels, Brussels Belgium
SOURCE: Applied Biochemistry and Biotechnology, (1994) Vol. 49, No. 1, pp. 75-91.
ISSN: 0273-2289.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Monomethoxypoly(ethylene glycol)-N-succinimide carbonate (SC-PEG) was used to prepare PEG-lysozyme, PEG-papaya proteinase 111, PEG-catalase, and PEG-lactoperoxidase conjugates. SC-PEG produced extensively modified enzymes under mild conditions (pH 7.0; 25 degree C) within a couple of hours. PEG-enzyme conjugates showed equal or even greater specific activity provided that low-molecular-weight substrates were used to evaluate the biological activities. However, papaya proteinase III and lysozyme lost their proteolytic and bacteriolytic activities, respectively, on conjugation with PEG. This was most probably because of steric factors, since no drastic conformational changes could be detected after conjugation of these enzymes with PEG chains. Unlike these enzymes, the secondary structures of the two hemoproteins were somewhat affected by the covalent attachment of PEG chains as shown by FTIR experiments. These results confirmed the potential usefulness of SC-PEG, for which a novel route of synthesis making use of N,N'-disuccinimidyl carbonate was described.

L15 ANSWER 12 OF 16 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 93289947 MEDLINE
DOCUMENT NUMBER: 93289947 PubMed ID: 8512060
TITLE: Reagents for the preparation of chromophorically labeled polyethylene glycol-protein conjugates.
AUTHOR: Ladd D L; Snow R A
CORPORATE SOURCE: Medicinal Chemistry Department, Sterling Winthrop Pharmaceuticals Research Division, Sterling Winthrop Inc., Malvern, Pennsylvania 19355.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1993 May 1) 210 (2) 258-61. Journal code: 4NK; 0370535. ISSN: 0003-2697.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 19930723
Entered Medline: 19930709

AB We have developed a new class of reagents (2) for the **covalent** attachment of **polyethylene glycol** to proteins. These reagents (2) are the monomethoxypolyethylene glycol esters of 4-fluoro-3-nitrobenzoic acid. The reaction of 2 with lysine epsilon-amino groups produces a chromophore which can be used to quantitate the **polyethylene glycol** to protein molar ratio. Bovine (Zn, Cu) **superoxide dismutase** was used as a model protein for conjugation with 2. When monomethoxypolyethylene glycol of average **molecular weight** 2105 was used, a conjugate was obtained with a **polyethylene glycol** to protein molar ratio of 8.88 retaining 100% of native enzymatic activity; monomethoxypolyethylene glycol of average **molecular weight** 5210 yielded a conjugate with a **polyethylene glycol** to protein molar ratio of 9.96 retaining 73% of native enzymatic activity.

L15 ANSWER 13 OF 16 MEDLINE DUPLICATE 5

ACCESSION NUMBER: 90339014 MEDLINE
DOCUMENT NUMBER: 90339014 PubMed ID: 2166134
TITLE: Spectroscopic characterization of polyethyleneglycol modified **superoxide dismutase**: 1H NMR studies on its Cu₂Co₂ derivative.
AUTHOR: Banci L; Bertini I; Caliceti P; Monsu Scolaro L; Schiavon O; Veronese F M
CORPORATE SOURCE: Department of Chemistry, University of Florence, Italy.
SOURCE: JOURNAL OF INORGANIC BIOCHEMISTRY, (1990 Jun) 39 (2) 149-59.
Journal code: JAR; 7905788. ISSN: 0162-0134.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901012
Last Updated on STN: 19901012
Entered Medline: 19900910

AB Spectroscopic methods have been employed in order to understand the molecular basis of the decrease in enzymatic activity of the antiinflammatory enzyme copper-zinc **superoxide dismutase** (SOD) following the **covalent** binding of polyethyleneglycol (PEG) chains to the protein amino-groups. The PEG modification is a general method recently proposed to improve the therapeutic index of enzymes. 1H NMR spectra on the cobalt substituted PEG-modified SOD, Cu₂Co₂-PEG-SOD, have been recorded. The signals are quite broad with respect to the unmodified enzyme. This has been interpreted on the basis of the effect of **molecular weight** on the linewidth. The analysis has shown that the histidine hydrogens involved in metal binding at the enzyme active site are the same in both native and PEG-modified SOD. Similarly, circular dichroism and absorption spectra indicate that the overall conformation of the metal clusters is not perturbed upon modification. On the other hand, azide titration shows that the affinity constant of N-3 for SOD is largely reduced upon PEG modification (K = 154 M⁻¹ and 75 M⁻¹ for the native and modified SOD, respectively). These results indicate that the decrease in enzymatic activity upon surface modification with PEG is not caused by a perturbation of the active site geometry, but to a

decrease in the channeling of the O₂- ion towards the enzyme active site.

L15 ANSWER 14 OF 16 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 6
ACCESSION NUMBER: 1988:507428 BIOSIS
DOCUMENT NUMBER: BA86:128112
TITLE: ANALYSIS OF **POLYETHYLENE GLYCOL**
MODIFIED **SUPEROXIDE DISMUTASE** BY
CHROMATOGRAPHIC ELECTROPHORETIC LIGHT SCATTERING CHEMICAL
AND ENZYMATIC METHODS.
AUTHOR(S): MCGOFF P; BAZIOTIS A C; MASKIEWICZ R
CORPORATE SOURCE: BOEHRINGER INGELLHEIM PHARMACEUTICALS INC., RIDGEFIELD,
CONN. 06877, USA.
SOURCE: CHEM PHARM BULL (TOKYO), (1988) 36 (8), 3079-3091.
CODEN: CPBTAL. ISSN: 0009-2363.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB **Covalent** conjugation of bovine erythrocyte **superoxide dismutase** (SOD) with activated **polyethylene glycol** (PEG) results in a mixture of modified species (PEG-SOD) with properties different from those of the native enzyme. The components of this mixture were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing, and chromatographic (size-exclusion, anion-exchange, cation-exchange and reverse-phase high performance liquid chromatography) techniques. Physicochemical properties such as apparent **molecular weight**, isoelectric point, relative hydrophobicity and relative cation-anion charge number were measured by electrophoretic and chromatographic procedures. Dispersity and apparent radius were examined by chromatographic and light scattering techniques. The extent of **covalent** modification and enzymatic activity change were measured by chemical and spectroscopic methods, showing that activity loss was not due to catalytic site modification. The properties of the PEG-modified form of the enzyme were compared with those of native SOD and showed that in addition to changing biological properties, PEG modification of proteins can result in a product with unexpectedly high heterogeneity and substantial changes in isoelectric point and hydrophobicity. Altered biological properties may therefore not merely be due to shielding of protein surface by PEG chains. Apparent properties of PEG modified proteins such as **molecular weight** were found to be highly method dependent, with poor agreement being shown among classical measurements.

L15 ANSWER 15 OF 16 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1979:451927 HCAPLUS
DOCUMENT NUMBER: 91:51927
TITLE: Preparation and properties of **polyethylene glycol**-trypsin adducts
AUTHOR(S): Abuchowski, Abraham; Davis, Frank F.
CORPORATE SOURCE: Bur. Biol. Res., Rutgers, State Univ., New Brunswick, NJ, 08903, USA
SOURCE: Biochim. Biophys. Acta (1979), 578(1), 41-6
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The **covalent** attachment of **polyethylene glycol** (mol. wt., 5000 daltons) to nonessential groups on trypsin produced an adduct that no longer pptd. with antitrypsin antibody.

In comparison with trypsin, **polyethylene glycol**-trypsin preps. showed equal or greater activity against N-.alpha.-benzoyl-L-arginine Et ester, .apprx.1/4 activity against angiotensin II, and little activity against bovine liver **catalase**. The adduct dissolved soft blood clots at 1/4 the rate of trypsin. Soybean trypsin inhibitor produced 2/3 inhibition of the adduct under conditions that caused complete inhibition of trypsin.

L15 ANSWER 16 OF 16 MEDLINE
 ACCESSION NUMBER: 7848 MEDLINE
 DOCUMENT NUMBER: 77187848 PubMed ID: 16907
 TITLE: Effect of **covalent** attachment of
polyethylene glycol on immunogenicity and
 circulating life of bovine liver **catalase**.
 AUTHOR: Abuchowski A; McCoy J R; Palczuk N C; van Es T; Davis F F
 SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1977 Jun 10) 252 (11)
 3582-6.
 Journal code: HIV; 2985121R. ISSN: 0021-9258.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 197707
 ENTRY DATE: Entered STN: 19900314
 Last Updated on STN: 19950206
 Entered Medline: 19770723

AB Methoxypolyethylene glycols of 1900 daltons (**PEG-1900**) or 5000 daltons (**PEG-5000**) were covalently attached to bovine liver **catalase** using 2,4,6-trichloro-s-triazine as the coupling agent. Rabbits were immunized by the intravenous and intramuscular routes with **catalase** modified by **covalent** attachment of **PEG-1900** to 43% of the amino groups (**PEG-1900-catalase**). The intravenous antiserum did not yield detectable antibodies against **PEG-1900-catalase** or native **catalase**, as determined by Ouchterlony and complement fixation methods, whereas the intramuscular antiserum contained antibodies to both **PEG-1900-catalase** and **catalase**. **PEG-1900** did not react with either antiserum. **Catalase** was prepared in which **PEG-5000** was attached to 40% of the amino groups (**PEG-5000-catalase**). This **catalase** preparation did not react with either antiserum. **PEG-1900-catalase** retained 93% of its enzymatic activity; **PEG-5000-catalase** retained 95%. **PEG-5000-catalase** resisted digestion by trypsin, chymotrypsin, and a protease from *Streptomyces griseus*. **PEG-1900-catalase** and **PEG-5000-catalase** exhibited enhanced circulating lives in the blood of acatalasemic mice during repetitive intravenous injections. No evidence was seen of an immune response to injections of the modified enzymes. Mice injected repetitively with **PEG-5000-catalase** remained immune competent for unmodified **catalase**, and no evidence of tissue or organ damage was seen.

=> d his

(FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001

L1 60112 S PEG
 L2 0 S POLYETHYLENE ADJ GLYCOL
 L3 110582 S POLYETHYLENE (A) GLYCOL
 L4 146676 S L1 OR L3
 L5 885718 S MOLECULAR(W) WEIGHT
 L6 40836 S "8000" OR "10000" OR "18000"
 L7 5333 S L5 AND L6
 L8 576 S L4 AND L7
 L9 124212 S COVALENT OR IMMOBLI?
 L10 247404 S (SUPEROXIDE (A)DISMUTASE?) OR CATALASE? OR (GLUTATHIONE(A)
 PE
 L11 1277 S L9 AND L10
 L12 0 S L8 AND L11
 L13 113 S L11 AND L4

L14 30 S L5 AND L13
L15 16 DUP REM L14 (14 DUPLICATES REMOVED)
L16 2362944 S WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?
L17 0 S L15 AND L16

=> s isocyanate?

L18 70993 ISOCYANATE?

=> s l18 and l15

L19 0 L18 AND L15

=> s diisocyanate?

L20 48865 DIISOCYANATE?

=> s l15 and l20

L21 0 L15 AND L20

=> d his

(FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001

L1 60112 S PEG
L2 0 S POLYETHYLENE ADJ GLYCOL
L3 110582 S POLYETHYLENE (A) GLYCOL
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L6 40836 S "8000" OR "10000" OR "18000"
L7 5333 S L5 AND L6
L8 576 S L4 AND L7
L9 124212 S COVALENT OR IMMOBILI?
L10 247404 S (SUPEROXIDE (A)DISMUTASE?) OR CATALASE? OR (GLUTATHIONE(A)
PE
L11 1277 S L9 AND L10
L12 0 S L8 AND L11
L13 113 S L11 AND L4
L14 30 S L5 AND L13
L15 16 DUP REM L14 (14 DUPLICATES REMOVED)
L16 2362944 S WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?
L17 0 S L15 AND L16
L18 70993 S ISOCYANATE?
L19 0 S L18 AND L15
L20 48865 S DIISOCYANATE?
L21 0 S L15 AND L20

=> s l15 and (urea or urethane?)

L22 0 L15 AND (UREA OR URETHANE?)

=> s l15 and amino

L23 6 L15 AND AMINO

=> dup rem l23

PROCESSING COMPLETED FOR L23

L24 6 DUP REM L23 (0 DUPLICATES REMOVED)

=> d 1-6 ibib ab

L24 ANSWER 1 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)

ACCESSION NUMBER: 1998:12669 SCISEARCH

THE GENUINE ARTICLE: BK03M

TITLE: Conjugation of high-molecular weight
poly(ethylene glycol) to cytokines:

Granulocyte-macrophage

colony-stimulating factors as model substrates

AUTHOR: Sherman M R (Reprint); Williams L D; Saifer M G P; French
J A; Kwak L W; Oppenheim J J

CORPORATE SOURCE: MT VIEW PHARMACEUT INC, 871-L IND PK, MENLO PK, CA 94025
(Reprint); NCI, FREDERICK CANC RES & DEV CTR, FREDERICK,
MD 21702

COUNTRY OF AUTHOR: USA

SOURCE: ACS SYMPOSIUM SERIES, (FEB 1997) Vol. 680, pp. 155-169.
Publisher: AMER CHEMICAL SOC, 1155 SIXTEENTH ST NW,
WASHINGTON, DC 20036.
ISSN: 0097-6156.

DOCUMENT TYPE: General Review; Journal

LANGUAGE: English

REFERENCE COUNT: 71

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The ability of the small receptor-binding protein, recombinant murine
granulocyte-macrophage colony-stimulating factor (GM-CSF), to increase
the

abundance of certain blood cell types in mice was enhanced markedly by
covalent attachment of a single long strand of **PEG**
(30-40 kDa). Potency was not increased further by coupling a second
strand. Such conjugates can be synthesized efficiently by reaction of
protein **amino** groups with **PEG** propionaldehydes in the
presence of NaBH₃CN or with **PEG** p-nitrophenyl carbonates. Both
methods have been used to prepare recombinant human GM-CSF conjugates of
predetermined composition, e.g. **PEG**(1)GM-CSF and **PEG**
(2)GM-CSF, in high yield. These compounds, or analogous derivatives of
other cytokines, purified by ion-exchange and size-exclusion
chromatography, may be suitable candidates for pharmaceutical
development.

L24 ANSWER 2 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)

ACCESSION NUMBER: 97:222875 SCISEARCH

THE GENUINE ARTICLE: WN017

TITLE: A simple and efficient method for preparation of
monomethoxypolyethylene glycol activated with
p-nitrophenylchloroformate and its application to
modification of L-asparaginase

AUTHOR: Kito M; Miron T; Wilchek M; Kojima N; Ohishi N; Yagi K
(Reprint)

CORPORATE SOURCE: INST APPL BIOCHEM, YAGI MEM PK, GIFU 50501, JAPAN
(Reprint); INST APPL BIOCHEM, GIFU 50501, JAPAN; WEIZMANN
INST SCI, DEPT BIOPHYS, IL-76100 REHOVOT, ISRAEL

COUNTRY OF AUTHOR: JAPAN; ISRAEL

SOURCE: JOURNAL OF CLINICAL BIOCHEMISTRY AND NUTRITION, (SEP
1996)

Vol. 21, No. 2, pp. 101-111.

Publisher: INST APPLIED BIOCHEMISTRY, YAGI MEMORIAL PARK,
MITAKE GIFU 505-01, JAPAN.

ISSN: 0912-0009.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 28

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An improved, simple and efficient method for preparation of
monomethoxypolyethylene glycol (**PEG**) activated with
p-nitrophenylchloroformate (PNP-**PEG**) and its use as a potent
modifier of protein under mild conditions are described. Modification of

bovine serum albumin with PNP-PEG was compared with that done with PEG activated with N,N'-carbonyldiimidazole or cyanuric chloride. The reaction of PEG, activated with either p-nitrophenylchloroformate or cyanuric chloride, with bovine serum albumin at 4 degrees C reached a plateau within 1 h, whereas protein modification using PEG activated with N,N'-carbonyldiimidazole was rather slow and gave a low yield. The remaining activity of L-asparaginase modified with PNP-PEG was much higher than that of the enzyme modified to the same degree with PEG activated with cyanuric chloride. At a 20 molar excess of PNP-PEG having a molecular weight of 5,000, 55% of the free amino acid groups were modified at 4 degrees C for 2 h, and the modified enzyme still had 33% residual enzyme activity. Immunochemical studies showed that the highly modified enzyme (67% modification with 18% residual enzyme activity) had lost its immunogenicity and had become much less sensitive to protease digestion.

L24 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
 ACCESSION NUMBER: 1995:16065 BIOSIS
 DOCUMENT NUMBER: PREV199598030365
 TITLE: Acylation of amino functions of proteins with monomethoxypoly (ethylene glycol)-N-succinimide carbonate.
 AUTHOR(S): Nijs, Michelle; Gelbcke, Michel; Azarkan, Mohamed; Brygier, Jeanne; Guermant, Claude; Baeyens-Volant, Danielle; Musu, Tony; Paul, Claudine; Looze, Yvan (1)
 CORPORATE SOURCE: (1) Protein Chem. Unit, Fac. Med., Univ. Brussels, Brussels Belgium
 SOURCE: Applied Biochemistry and Biotechnology, (1994) Vol. 49, No. 1, pp. 75-91.
 ISSN: 0273-2289.
 DOCUMENT TYPE: Article
 LANGUAGE: English
 AB Monomethoxypoly(ethylene glycol)-N-succinimide carbonate (SC-PEG) was used to prepare PEG-lysozyme, PEG-papaya proteinase 111, PEG-catalase, and PEG-lactoperoxidase conjugates. SC-PEG produced extensively modified enzymes under mild conditions (pH 7.0; 25 degree C) within a couple of hours. PEG-enzyme conjugates showed equal or even greater specific activity provided that low-molecular-weight substrates were used to evaluate the biological activities. However, papaya proteinase III and lysozyme lost their proteolytic and bacteriolytic activities, respectively, on conjugation with PEG. This was most probably because of steric factors, since no drastic conformational changes could be detected after conjugation of these enzymes with PEG chains. Unlike these enzymes, the secondary structures of the two hemoproteins were somewhat affected by the covalent attachment of PEG chains as shown by FTIR experiments. These results confirmed the potential usefulness of SC-PEG, for which a novel route of synthesis making use of N,N'-disuccinimidyl carbonate was described.

L24 ANSWER 4 OF 6 MEDLINE
 ACCESSION NUMBER: 93289947 MEDLINE
 DOCUMENT NUMBER: 93289947 PubMed ID: 8512060
 TITLE: Reagents for the preparation of chromophorically labeled polyethylene glycol-protein conjugates.
 AUTHOR: Ladd D L; Snow R A
 CORPORATE SOURCE: Medicinal Chemistry Department, Sterling Winthrop Pharmaceuticals Research Division, Sterling Winthrop Inc., Malvern, Pennsylvania 19355.

SOURCE: ANALYTICAL BIOCHEMISTRY, (1993 May 1) 210 (2) 258-61.
Journal code: 4NK; 0370535. ISSN: 03-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 19930723
Entered Medline: 19930709

AB We have developed a new class of reagents (2) for the **covalent** attachment of **polyethylene glycol** to proteins. These reagents (2) are the monomethoxypolyethylene glycol esters of 4-fluoro-3-nitrobenzoic acid. The reaction of 2 with lysine epsilon-**amino** groups produces a chromophore which can be used to quantitate the **polyethylene glycol** to protein molar ratio. Bovine (Zn, Cu) **superoxide dismutase** was used as a model protein for conjugation with 2. When monomethoxypolyethylene glycol of average **molecular weight** 2105 was used, a conjugate was obtained with a **polyethylene glycol** to protein molar ratio of 8.88 retaining 100% of native enzymatic activity; monomethoxypolyethylene glycol of average **molecular weight** 5210 yielded a conjugate with a **polyethylene glycol** to protein molar ratio of 9.96 retaining 73% of native enzymatic activity.

L24 ANSWER 5 OF 6 MEDLINE

ACCESSION NUMBER: 90339014 MEDLINE

DOCUMENT NUMBER: 90339014 PubMed ID: 2166134

TITLE: Spectroscopic characterization of polyethyleneglycol modified **superoxide dismutase**: 1H NMR studies on its Cu₂Co₂ derivative.

AUTHOR: Banci L; Bertini I; Caliceti P; Monsu Scolaro L; Schiavon O; Veronese F M

CORPORATE SOURCE: Department of Chemistry, University of Florence, Italy.

SOURCE: JOURNAL OF INORGANIC BIOCHEMISTRY, (1990 Jun) 39 (2) 149-59.

Journal code: JAR; 7905788. ISSN: 0162-0134.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199009

ENTRY DATE: Entered STN: 19901012

Last Updated on STN: 19901012

Entered Medline: 19900910

AB Spectroscopic methods have been employed in order to understand the molecular basis of the decrease in enzymatic activity of the antiinflammatory enzyme copper-zinc **superoxide dismutase** (SOD) following the **covalent** binding of polyethyleneglycol (**PEG**) chains to the protein **amino**-groups. The **PEG** modification is a general method recently proposed to improve the therapeutic index of enzymes. 1H NMR spectra on the cobalt substituted **PEG**-modified SOD, Cu₂Co₂-**PEG**-SOD, have been recorded. The signals are quite broad with respect to the unmodified enzyme. This has been interpreted on the basis of the effect of **molecular weight** on the linewidth. The analysis has shown that the histidine hydrogens involved in metal binding at the enzyme active site are the same in both native and **PEG**-modified SOD. Similarly, circular dichroism and absorption spectra indicate that the overall conformation of the metal clusters is not perturbed upon modification. On the other hand, azide titration shows that the affinity constant of N-3 for SOD is largely reduced upon **PEG** modification (K = 154 M⁻¹ and 75 M⁻¹ for the

native and modified SOD, respectively). These results indicate that the decrease in enzymic activity upon surface modification with PEG is not caused by a perturbation of the active site geometry, but to a decrease in the channeling of the O₂- ion towards the enzyme active site.

L24 ANSWER 6 OF 6 MEDLINE
 ACCESSION NUMBER: 77187848 MEDLINE
 DOCUMENT NUMBER: 77187848 PubMed ID: 16907
 TITLE: Effect of **covalent** attachment of **polyethylene glycol** on immunogenicity and circulating life of bovine liver **catalase**.
 AUTHOR: Abuchowski A; McCoy J R; Palczuk N C; van Es T; Davis F F
 SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1977 Jun 10) 252 (11) 3582-6.
 Journal code: HIV; 2985121R. ISSN: 0021-9258.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 197707
 ENTRY DATE: Entered STN: 19900314
 Last Updated on STN: 19950206
 Entered Medline: 19770723

AB Methoxypolyethylene glycols of 1900 daltons (PEG-1900) or 5000 daltons (PEG-5000) were covalently attached to bovine liver **catalase** using 2,4,6-trichloro-s-triazine as the coupling agent. Rabbits were immunized by the intravenous and intramuscular routes with **catalase** modified by **covalent** attachment of PEG-1900 to 43% of the **amino** groups (PEG-1900-**catalase**). The intravenous antiserum did not yield detectable antibodies against PEG-1900-**catalase** or native **catalase**, as determined by Ouchterlony and complement fixation methods, whereas the intramuscular antiserum contained antibodies to both PEG-1900-**catalase** and **catalase**. PEG-1900 did not react with either antiserum. **Catalase** was prepared in which PEG-5000 was attached to 40% of the **amino** groups (PEG-5000-**catalase**). This **catalase** preparation did not react with either antiserum. PEG-1900-**catalase** retained 93% of its enzymatic activity; PEG-5000-**catalase** retained 95%. PEG-5000-**catalase** resisted digestion by trypsin, chymotrypsin, and a protease from *Streptomyces griseus*. PEG-1900-**catalase** and PEG-5000-**catalase** exhibited enhanced circulating lives in the blood of acatalasemic mice during repetitive intravenous injections. No evidence was seen of an immune response to injections of the modified enzymes. Mice injected repetitively with PEG-5000-**catalase** remained immune competent for unmodified **catalase**, and no evidence of tissue or organ damage was seen.

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(FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001

L1 60112 S PEG
 L2 0 S POLYETHYLENE ADJ GLYCOL
 L3 110582 S POLYETHYLENE (A) GLYCOL
 L4 146676 S L1 OR L3
 L5 885718 S MOLECULAR(W) WEIGHT
 L6 40836 S "8000" OR "10000" OR "18000"
 L7 5333 S L5 AND L6
 L8 576 S L4 AND L7
 L9 124212 S COVALENT OR IMMOBILI?

L10 247404 S (SUPEROXIDE (A)DISMUTASE?) OR CATALASE? OR (GLUTATHIONE (A)
 PE
 L11 1277 S L9 AND L10
 L12 0 S L8 AND L11
 L13 113 S L11 AND L4
 L14 30 S L5 AND L13
 L15 16 DUP REM L14 (14 DUPLICATES REMOVED)
 L16 2362944 S WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?
 L17 0 S L15 AND L16
 L18 70993 S ISOCYANATE?
 L19 0 S L18 AND L15
 L20 48865 S DIISOCYANATE?
 L21 0 S L15 AND L20
 L22 0 S L15 AND (UREA OR URETHANE?)
 L23 6 S L15 AND AMINO
 L24 6 DUP REM L23 (0 DUPLICATES REMOVED)

=> e ettner n/au

E1 1 ETTNER H U F/AU
 E2 1 ETTNER I/AU
 E3 26 --> ETTNER N/AU
 E4 20 ETTNER NORBERT/AU
 E5 3 ETTNER S/AU
 E6 89 ETTNER S L/AU
 E7 3 ETTNER SUSAN L/AU
 E8 1 ETTNER SUSAN LOUISE/AU
 E9 5 ETTNER U/AU
 E10 1 ETTNGER B/AU
 E11 1 ETTNIQUI A/AU
 E12 4 ETTORE A/AU

=> s e3

L25 26 "ETTNER N"/AU

=> s l25 and l3

L26 0 L25 AND L3

=> s l24 and l4

L27 6 L24 AND L4

=> dup rem l27

PROCESSING COMPLETED FOR L27

L28 6 DUP REM L27 (0 DUPLICATES REMOVED)

=> d 1-6 ibib ab

L28 ANSWER 1 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)

ACCESSION NUMBER: 1998:12669 SCISEARCH

THE GENUINE ARTICLE: BK03M

TITLE: Conjugation of high-molecular weight
 poly(ethylene glycol) to cytokines:

Granulocyte-macrophage

colony-stimulating factors as model substrates

AUTHOR: Sherman M R (Reprint); Williams L D; Saifer M G P; French
 J A; Kwak L W; Oppenheim J J

CORPORATE SOURCE: MT VIEW PHARMACEUT INC, 871-L IND PK, MENLO PK, CA 94025
 (Reprint); NCI, FREDERICK CANC RES & DEV CTR, FREDERICK,
 MD 21702

COUNTRY OF AUTHOR: USA

SOURCE: ACS SYMPOSIUM SERIES, (FEB 1997) Vol. 680, pp. 155-169.

DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 71

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The ability of the small receptor-binding protein, recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF), to increase the abundance of certain blood cell types in mice was enhanced markedly by **covalent** attachment of a single long strand of **PEG** (30-40 kDa). Potency was not increased further by coupling a second strand. Such conjugates can be synthesized efficiently by reaction of protein **amino** groups with **PEG** propionaldehydes in the presence of NaBH₃CN or with **PEG** p-nitrophenyl carbonates. Both methods have been used to prepare recombinant human GM-CSF conjugates of predetermined composition, e.g. **PEG**(1)GM-CSF and **PEG**(2)GM-CSF, in high yield. These compounds, or analogous derivatives of other cytokines, purified by ion-exchange and size-exclusion chromatography, may be suitable candidates for pharmaceutical development.

L28 ANSWER 2 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)

ACCESSION NUMBER: 97:222875 SCISEARCH

THE GENUINE ARTICLE: WN017

TITLE: A simple and efficient method for preparation of monomethoxypolyethylene glycol activated with p-nitrophenylchloroformate and its application to modification of L-asparaginase

AUTHOR: Kito M; Miron T; Wilchek M; Kojima N; Ohishi N; Yagi K (Reprint)

CORPORATE SOURCE: INST APPL BIOCHEM, YAGI MEM PK, GIFU 50501, JAPAN (Reprint); INST APPL BIOCHEM, GIFU 50501, JAPAN; WEIZMANN INST SCI, DEPT BIOPHYS, IL-76100 REHOVOT, ISRAEL

COUNTRY OF AUTHOR: JAPAN; ISRAEL

SOURCE: JOURNAL OF CLINICAL BIOCHEMISTRY AND NUTRITION, (SEP 1996)

Vol. 21, No. 2, pp. 101-111.

Publisher: INST APPLIED BIOCHEMISTRY, YAGI MEMORIAL PARK, MITAKE GIFU 505-01, JAPAN.

ISSN: 0912-0009.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 28

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An improved, simple and efficient method for preparation of monomethoxypolyethylene glycol (**PEG**) activated with p-nitrophenylchloroformate (PNP-**PEG**) and its use as a potent modifier of protein under mild conditions are described. Modification of bovine serum albumin with PNP-**PEG** was compared with that done with **PEG** activated with N,N'-carbonyldiimidazole or cyanuric chloride. The reaction of **PEG**, activated with either p-nitrophenylchloroformate or cyanuric chloride, with bovine serum albumin at 4 degrees C reached a plateau within 1 h, whereas protein modification using **PEG** activated with N,N'-carbonyldiimidazole was rather slow and gave a low yield. The remaining activity of L-asparaginase modified with PNP-**PEG** was much higher than that of the enzyme modified to the same degree with **PEG** activated with cyanuric chloride. At a 20 molar excess of PNP-**PEG** having a **molecular weight** of 5,000, 55% of the free **amino** acid groups were modified at 4 degrees C for 2 h, and the modified enzyme still had 33% residual enzyme activity. Immunochemical studies showed that

the highly modified enzyme (67% modification with 18% residual enzyme activity) had lost its immunogenicity and had become much less sensitive to protease digestion.

L28 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1995:16065 BIOSIS
DOCUMENT NUMBER: PREV199598030365
TITLE: Acylation of **amino** functions of proteins with monomethoxypoly (ethylene glycol)-N-succinimide carbonate.
AUTHOR(S): Nijs, Michelle; Gelbcke, Michel; Azarkan, Mohamed; Brygier, Jeanne; Guermant, Claude; Baeyens-Volant, Danielle; Musu, Tony; Paul, Claudine; Looze, Yvan (1)
CORPORATE SOURCE: (1) Protein Chem. Unit, Fac. Med., Univ. Brussels, Brussels
SOURCE: Applied Biochemistry and Biotechnology, (1994) Vol. 49, No. 1, pp. 75-91.
ISSN: 0273-2289.

DOCUMENT TYPE: Article
LANGUAGE: English

AB Monomethoxypoly(ethylene glycol)-N-succinimide carbonate (SC-**PEG**) was used to prepare **PEG**-lysozyme, **PEG**-papaya proteinase 111, **PEG**-catalase, and **PEG**-lactoperoxidase conjugates. SC-**PEG** produced extensively modified enzymes under mild conditions (pH 7.0; 25 degree C) within a couple of hours. **PEG**-enzyme conjugates showed equal or even greater specific activity provided that low-molecular-weight substrates were used to evaluate the biological activities. However, papaya proteinase III and lysozyme lost their proteolytic and bacteriolytic activities, respectively, on conjugation with **PEG**. This was most probably because of steric factors, since no drastic conformational changes could be detected after conjugation of these enzymes with **PEG** chains. Unlike these enzymes, the secondary structures of the two hemoproteins were somewhat affected by the **covalent** attachment of **PEG** chains as shown by FTIR experiments. These results confirmed the potential usefulness of SC-**PEG**, for which a novel route of synthesis making use of N,N'-disuccinimidyl carbonate was described.

L28 ANSWER 4 OF 6 MEDLINE
ACCESSION NUMBER: 93289947 MEDLINE
DOCUMENT NUMBER: 93289947 PubMed ID: 8512060
TITLE: Reagents for the preparation of chromophorically labeled **polyethylene glycol**-protein conjugates.
AUTHOR: Ladd D L; Snow R A
CORPORATE SOURCE: Medicinal Chemistry Department, Sterling Winthrop Pharmaceuticals Research Division, Sterling Winthrop Inc., Malvern, Pennsylvania 19355.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1993 May 1) 210 (2) 258-61. Journal code: 4NK; 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 19930723
Entered Medline: 19930709

AB We have developed a new class of reagents (2) for the **covalent** attachment of **polyethylene glycol** to proteins. These reagents (2) are the monomethoxypolyethylene glycol esters of 4-fluoro-3-nitrobenzoic acid. The reaction of 2 with lysine epsilon-**amino** groups produces a chromophore which can be used to

quantitate the **polyethylene glycol** to protein molar ratio. Bovine (2 Cu) **superoxide dismutase** was used as a model protein for conjugation with 2. When monomethoxypolyethylene glycol of average **molecular weight** 2105 was used, a conjugate was obtained with a **polyethylene glycol** to protein molar ratio of 8.88 retaining 100% of native enzymatic activity; monomethoxypolyethylene glycol of average **molecular weight** 5210 yielded a conjugate with a **polyethylene glycol** to protein molar ratio of 9.96 retaining 73% of native enzymatic activity.

L28 ANSWER 5 OF 6 MEDLINE
 ACCESSION NUMBER: 90339014 MEDLINE
 DOCUMENT NUMBER: 90339014 PubMed ID: 2166134
 TITLE: Spectroscopic characterization of polyethyleneglycol modified **superoxide dismutase**: 1H NMR studies on its Cu₂Co₂ derivative.
 AUTHOR: Banci L; Bertini I; Caliceti P; Monsu Scolaro L; Schiavon O; Veronese F M
 CORPORATE SOURCE: Department of Chemistry, University of Florence, Italy.
 SOURCE: JOURNAL OF INORGANIC BIOCHEMISTRY, (1990 Jun) 39 (2) 149-59.
 Journal code: JAR; 7905788. ISSN: 0162-0134.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199009
 ENTRY DATE: Entered STN: 19901012
 Last Updated on STN: 19901012
 Entered Medline: 19900910

AB Spectroscopic methods have been employed in order to understand the molecular basis of the decrease in enzymatic activity of the antiinflammatory enzyme copper-zinc **superoxide dismutase** (SOD) following the **covalent** binding of polyethyleneglycol (PEG) chains to the protein amino-groups. The PEG modification is a general method recently proposed to improve the therapeutic index of enzymes. 1H NMR spectra on the cobalt substituted PEG-modified SOD, Cu₂Co₂-PEG-SOD, have been recorded. The signals are quite broad with respect to the unmodified enzyme. This has been interpreted on the basis of the effect of **molecular weight** on the linewidth. The analysis has shown that the histidine hydrogens involved in metal binding at the enzyme active site are the same in both native and PEG-modified SOD. Similarly, circular dichroism and absorption spectra indicate that the overall conformation of the metal clusters is not perturbed upon modification. On the other hand, azide titration shows that the affinity constant of N-3 for SOD is largely reduced upon PEG modification (K = 154 M⁻¹ and 75 M⁻¹ for the native and modified SOD, respectively). These results indicate that the decrease in enzymatic activity upon surface modification with PEG is not caused by a perturbation of the active site geometry, but to a decrease in the channeling of the O₂⁻ ion towards the enzyme active site.

L28 ANSWER 6 OF 6 MEDLINE
 ACCESSION NUMBER: 77187848 MEDLINE
 DOCUMENT NUMBER: 77187848 PubMed ID: 16907
 TITLE: Effect of **covalent** attachment of **polyethylene glycol** on immunogenicity and circulating life of bovine liver **catalase**.
 AUTHOR: Abuchowski A; McCoy J R; Palczuk N C; van Es T; Davis F F
 SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1977 Jun 10) 252 (11) 3582-6.
 Journal code: HIV; 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 197707
 ENTRY DATE: Entered STN: 19900314
 Last Updated on STN: 19950206
 Entered Medline: 19770723

AB Methoxypolyethylene glycols of 1900 daltons (PEG-1900) or 5000 daltons (PEG-5000) were covalently attached to bovine liver **catalase** using 2,4,6-trichloro-s-triazine as the coupling agent. Rabbits were immunized by the intravenous and intramuscular routes with **catalase** modified by covalent attachment of PEG-1900 to 43% of the amino groups (PEG-1900-**catalase**). The intravenous antiserum did not yield detectable antibodies against PEG-1900-**catalase** or native **catalase**, as determined by Ouchterlony and complement fixation methods, whereas the intramuscular antiserum contained antibodies to both PEG-1900-**catalase** and **catalase**. PEG-1900 did not react with either antiserum. **Catalase** was prepared in which PEG-5000 was attached to 40% of the amino groups (PEG-5000-**catalase**). This **catalase** preparation did not react with either antiserum. PEG-1900-**catalase** retained 93% of its enzymatic activity; PEG-5000-**catalase** retained 95%. PEG-5000-**catalase** resisted digestion by trypsin, chymotrypsin, and a protease from *Streptomyces griseus*. PEG-1900-**catalase** and PEG-5000-**catalase** exhibited enhanced circulating lives in the blood of acatalasemic mice during repetitive intravenous injections. No evidence was seen of an immune response to injections of the modified enzymes. Mice injected repetitively with PEG-5000-**catalase** remained immune competent for unmodified **catalase**, and no evidence of tissue or organ damage was seen.

=> d his

(FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001

L1 60112 S PEG
 L2 0 S POLYETHYLENE ADJ GLYCOL
 L3 110582 S POLYETHYLENE (A) GLYCOL
 L4 146676 S L1 OR L3
 L5 885718 S MOLECULAR(W) WEIGHT
 L6 40836 S "8000" OR "10000" OR "18000"
 L7 5333 S L5 AND L6
 L8 576 S L4 AND L7
 L9 124212 S COVALENT OR IMMOBLI?
 L10 247404 S (SUPEROXIDE (A)DISMUTASE?) OR CATALASE? OR (GLUTATHIONE(A)
 PE
 L11 1277 S L9 AND L10
 L12 0 S L8 AND L11
 L13 113 S L11 AND L4
 L14 30 S L5 AND L13
 L15 16 DUP REM L14 (14 DUPLICATES REMOVED)
 L16 2362944 S WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?
 L17 0 S L15 AND L16
 L18 70993 S ISOCYANATE?
 L19 0 S L18 AND L15
 L20 48865 S DIISOCYANATE?
 L21 0 S L15 AND L20
 L22 0 S L15 AND (UREA OR URETHANE?)
 L23 6 S L15 AND AMINO

```

L24          6 DUP REM L23 (0 DUPLICATES REMOVED)
              E ET1 N/AU
L25          26 S E3
L26          0 S L25 AND L3
L27          6 S L24 AND L4
L28          6 DUP REM L27 (0 DUPLICATES REMOVED)

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=> s l28 and l6

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L29          0 L28 AND L6

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=> e schink m/au

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E1           3      SCHINK KARL/AU
E2           2      SCHINK L/AU
E3          34 --> SCHINK M/AU
E4           1      SCHINK M J/AU
E5          10      SCHINK M M/AU
E6           3      SCHINK MAGDOLNA/AU
E7           1      SCHINK MAGDOLNA HORVAY/AU
E8           6      SCHINK MICHAEL/AU
E9           2      SCHINK MYRA/AU
E10          1      SCHINK N/AU
E11          46      SCHINK NORBERT/AU
E12          3      SCHINK NORBERT F/AU

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=> s e3

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L30          34 "SCHINK M"/AU

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=> s l4 and l30

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L31          0 L4 AND L30

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=> e schreiber J/au

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E1           2      SCHREIBER IRIS/AU
E2           2      SCHREIBER IRMELA/AU
E3          811 --> SCHREIBER J/AU
E4           4      SCHREIBER J A/AU
E5           37      SCHREIBER J B/AU
E6           8      SCHREIBER J C/AU
E7          116      SCHREIBER J D/AU
E8           8      SCHREIBER J E/AU
E9           12      SCHREIBER J F/AU
E10          3      SCHREIBER J F JR/AU
E11          60      SCHREIBER J H/AU
E12          7      SCHREIBER J J/AU

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=> s e3

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L32          811 "SCHREIBER J"/AU

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=> s l32 and l4

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L33          0 L32 AND L4

```

=> e meier w/au

```

E1           9      MEIER VOLKER/AU
E2           1      MEIER VOLKER VM/AU
E3          1206 --> MEIER W/AU
E4           45      MEIER W A/AU
E5           1      MEIER W B/AU
E6           3      MEIER W D/AU
E7           83      MEIER W E/AU

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| | | |
|-----|----|-----------------|
| E8 | 1 | MEIER W G/AU |
| E9 | 1 | MEIER W H/AU |
| E10 | 2 | MEIER W J/AU |
| E11 | 3 | MEIER W JEFF/AU |
| E12 | 24 | MEIER W L/AU |

=> s e3

L34 1206 "MEIER W"/AU

=> s l4 and l34

L35 3 L4 AND L34

=> dup rem l35

PROCESSING COMPLETED FOR L35

L36 2 DUP REM L35 (1 DUPLICATE REMOVED)

=> d 1-2 ibib ab

L36 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
 ACCESSION NUMBER: 2000:351159 BIOSIS
 DOCUMENT NUMBER: PREV200000351159
 TITLE: Treatment of progressive and recurrent ovarian cancer.
 AUTHOR(S): Meier, W. (1); Gropp, M.; Burges, A.; Hepp, H.
 CORPORATE SOURCE: (1) Frauenklinik, Evangelisches Krankenhaus,
 Kirchfeldstrasse 40, D-40217, Duesseldorf Germany
 SOURCE: Onkologie, (April, 2000) Vol. 23, No. Suppl. 2, pp. 35-39.
 print.
 ISSN: 0378-584X.
 DOCUMENT TYPE: Article
 LANGUAGE: German
 SUMMARY LANGUAGE: English; German
 AB Secondary surgery after failure of primary treatment is a promising and
 reasonable option only for patients with a relapse-free interval of at
 least 6-12 months who should have ideally achieved a tumor-free status
 after primary therapy. As after primary surgery, size of residual tumor
 is the most significant predictor of survival after secondary surgery. Even
 in the case of multiple tumor sites, complete removal of the tumor can be
 achieved in nearly 30% of the patients. Treatment results are much better
 in specialized oncology centers with optimal interdisciplinary
 cooperation compared with smaller institutions. Chemotherapy can be used both for
 consolidation after successful secondary surgery and for palliation in
 patients with inoperable recurrent disease. Since paclitaxel has been
 integrated into first-line chemotherapy, there is no defined standard for
 second-line chemotherapy. Several cytotoxic agents have shown moderate
 activity in this setting, including treosulfan, epirubicin, and newer
 agents such as topotecan, gemcitabine, vinorelbine, and PEG(
polyethylene glycol)-liposomal doxorubicin. Thus, the
 German Arbeitsgemeinschaft Gynaekologische Onkologie (AGO) has initiated
 several randomized studies in patients with recurrent ovarian cancer in
 order to define new standards for second-line chemotherapy.

L36 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2001 ACS
 ACCESSION NUMBER: 1997:502004 HCAPLUS
 DOCUMENT NUMBER: 127:177044
 TITLE: Structure of W/O-microemulsion-templated polymer
 networks
 AUTHOR(S): Meier, W.
 CORPORATE SOURCE: Institut Physikalische Chemie, Basel, CH-4056, Switz.
 SOURCE: Colloid Polym. Sci. (1997), 275(6), 530-536
 CODEN: CPMSB6; ISSN: 0303-402X

PUBLISHER: Steinkopff
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The droplet phase of a water-in-oil microemulsion is used as a matrix to create amphiphilic polymer network structures from a poly(oxyethylene)-poly(isoprene)-poly(oxyethylene) triblock copolymer bearing polymerizable methacrylate end groups. Cond. measurements and freeze fracture electron microscopy are used to obtain information about the structure of these gels. The droplet structure of the underlying microemulsion is only preserved for low droplet and polymer concn. For higher concns. the crosslinking reaction can induce the formation of a bicontinuous structure.

=> d 1-2 kwic

L36 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1

AU Meier, W. (1); Gropp, M.; Burges, A.; Hepp, H.

AB. . . agents have shown moderate activity in this setting, including treosulfan, epirubicin, and newer agents such as topotecan, gemcitabine, vinorelbine, and PEG(polyethylene glycol)-liposomal doxorubicin. Thus, the German Arbeitsgemeinschaft Gynaekologische Onkologie (AGO) has initiated several randomized studies in patients with recurrent ovarian cancer in. . .

IT . . .
disease, recurrence, reproductive system disease/female, treatment;
secondary surgery: surgical method, therapeutic method

IT Chemicals & Biochemicals
doxorubicin: antineoplastic - drug, polyethylene glycol-liposomal formulation; epirubicin: antineoplastic - drug; gemcitabine: antineoplastic - drug; paclitaxel: antineoplastic - drug; topotecan: antineoplastic - drug; treosulfan: antineoplastic -.

L36 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2001 ACS

AU Meier, W.

ST morphol polyethylene glycol polyisoprene microemulsion templated

=> e sauer M/au

| | | |
|-----|---------|------------------|
| E1 | 1 | SAUER LOUIS W/AU |
| E2 | 1 | SAUER LUDWIG/AU |
| E3 | 550 --> | SAUER M/AU |
| E4 | 34 | SAUER M A/AU |
| E5 | 1 | SAUER M B/AU |
| E6 | 81 | SAUER M C/AU |
| E7 | 28 | SAUER M C JR/AU |
| E8 | 6 | SAUER M C V/AU |
| E9 | 6 | SAUER M E/AU |
| E10 | 1 | SAUER M H M/AU |
| E11 | 176 | SAUER M J/AU |
| E12 | 25 | SAUER M K/AU |

=> s e3

L37 550 "SAUER M"/AU

=> s 14 and 137

L38 0 L4 AND L37

=> d his

(FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001

L1 60112 S PEG
L2 0 S POLYETHYLENE ADJ GLYCOL
L3 110582 S POLYETHYLENE (A) GLYCOL
L4 146676 S L1 OR L3
L5 885718 S MOLECULAR(W) WEIGHT
L6 40836 S "8000" OR "10000" OR "18000"
L7 5333 S L5 AND L6
L8 576 S L4 AND L7
L9 124212 S COVALENT OR IMMOBILI?
L10 247404 S (SUPEROXIDE (A) DISMUTASE?) OR CATALASE? OR (GLUTATHIONE(A)
PE
L11 1277 S L9 AND L10
L12 0 S L8 AND L11
L13 113 S L11 AND L4
L14 30 S L5 AND L13
L15 16 DUP REM L14 (14 DUPLICATES REMOVED)
L16 2362944 S WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?
L17 0 S L15 AND L16
L18 70993 S ISOCYANATE?
L19 0 S L18 AND L15
L20 48865 S DIISOCYANATE?
L21 0 S L15 AND L20
L22 0 S L15 AND (UREA OR URETHANE?)
L23 6 S L15 AND AMINO
L24 6 DUP REM L23 (0 DUPLICATES REMOVED)
E ETTNER N/AU
L25 26 S E3
L26 0 S L25 AND L3
L27 6 S L24 AND L4
L28 6 DUP REM L27 (0 DUPLICATES REMOVED)
L29 0 S L28 AND L6
E SCHINK M/AU
L30 34 S E3
L31 0 S L4 AND L30
E SCHREIBER J/AU
L32 811 S E3
L33 0 S L32 AND L4
E MEIER W/AU
L34 1206 S E3
L35 3 S L4 AND L34
L36 2 DUP REM L35 (1 DUPLICATE REMOVED)
E SAUER M/AU
L37 550 S E3
L38 0 S L4 AND L37

=> log hold

| | | |
|--|------------|---------|
| COST IN U.S. DOLLARS | SINCE FILE | TOTAL |
| | ENTRY | SESSION |
| FULL ESTIMATED COST | 126.41 | 126.86 |
| DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) | SINCE FILE | TOTAL |
| | ENTRY | SESSION |
| CA SUBSCRIBER PRICE | -1.76 | -1.76 |

SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 11:38:08 ON 08 NOV 2001

L21 0 S L15 AND L20
L22 0 S L15 AND (UREA OR URETHANE?)
L23 6 S L15 AND AMINO
L24 6 DUP REM L23 (0 DUPLICATES REMOVED)

=> e ettner n/au

E1 1 ETTNER H U F/AU
E2 1 ETTNER I/AU
E3 26 --> ETTNER N/AU
E4 20 ETTNER NORBERT/AU
E5 3 ETTNER S/AU
E6 89 ETTNER S L/AU
E7 3 ETTNER SUSAN L/AU
E8 1 ETTNER SUSAN LOUISE/AU
E9 5 ETTNER U/AU
E10 1 ETTNER B/AU
E11 1 ETTNIQUI A/AU
E12 4 ETTTORE A/AU

=> s e3

L25 26 "ETTNER N"/AU

=> s l25 and l3

L26 0 L25 AND L3

=> s l24 and l4

L27 6 L24 AND L4

=> dup rem l27

PROCESSING COMPLETED FOR L27

L28 6 DUP REM L27 (0 DUPLICATES REMOVED)

=> d 1-6 ibib ab

L28 ANSWER 1 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)

ACCESSION NUMBER: 1998:12669 SCISEARCH

THE GENUINE ARTICLE: BK03M

TITLE: Conjugation of high-molecular weight
poly(ethylene glycol) to cytokines:

Granulocyte-macrophage

colony-stimulating factors as model substrates

AUTHOR: Sherman M R (Reprint); Williams L D; Saifer M G P; French
J A; Kwak L W; Oppenheim J J

CORPORATE SOURCE: MT VIEW PHARMACEUT INC, 871-L IND PK, MENLO PK, CA 94025
(Reprint); NCI, FREDERICK CANC RES & DEV CTR, FREDERICK,
MD 21702

COUNTRY OF AUTHOR: USA

SOURCE: ACS SYMPOSIUM SERIES, (FEB 1997) Vol. 680, pp. 155-169.
Publisher: AMER CHEMICAL SOC, 1155 SIXTEENTH ST NW,
WASHINGTON, DC 20036.
ISSN: 0097-6156.

DOCUMENT TYPE: General Review; Journal

LANGUAGE: English

REFERENCE COUNT: 71

AB The ability of the small receptor-binding protein, recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF), to increase the abundance of certain blood cell types in mice was enhanced markedly by covalent attachment of a single long strand of PEG (30-40 kDa). Potency was not increased further by coupling a second strand. Such conjugates can be synthesized efficiently by reaction of protein amino groups with PEG propionaldehydes in the presence of NaBH₃CN or with PEG p-nitrophenyl carbonates. Both methods have been used to prepare recombinant human GM-CSF conjugates of predetermined composition, e.g. PEG(1)GM-CSF and PEG(2)GM-CSF, in high yield. These compounds, or analogous derivatives of other cytokines, purified by ion-exchange and size-exclusion chromatography, may be suitable candidates for pharmaceutical development.

L28 ANSWER 2 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)

ACCESSION NUMBER: 97:222875 SCISEARCH

THE GENUINE ARTICLE: WN017

TITLE: A simple and efficient method for preparation of monomethoxypolyethylene glycol activated with p-nitrophenylchloroformate and its application to modification of L-asparaginase

AUTHOR: Kito M; Miron T; Wilchek M; Kojima N; Ohishi N; Yagi K (Reprint)

CORPORATE SOURCE: INST APPL BIOCHEM, YAGI MEM PK, GIFU 50501, JAPAN (Reprint); INST APPL BIOCHEM, GIFU 50501, JAPAN; WEIZMANN INST SCI, DEPT BIOPHYS, IL-76100 REHOVOT, ISRAEL

COUNTRY OF AUTHOR: JAPAN; ISRAEL

SOURCE: JOURNAL OF CLINICAL BIOCHEMISTRY AND NUTRITION, (SEP 1996)

Vol. 21, No. 2, pp. 101-111.

Publisher: INST APPLIED BIOCHEMISTRY, YAGI MEMORIAL PARK, MITAKE GIFU 505-01, JAPAN.

ISSN: 0912-0009.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 28

AB An improved, simple and efficient method for preparation of monomethoxypolyethylene glycol (PEG) activated with p-nitrophenylchloroformate (PNP-PEG) and its use as a potent modifier of protein under mild conditions are described. Modification of bovine serum albumin with PNP-PEG was compared with that done with PEG activated with N,N'-carbonyldiimidazole or cyanuric chloride. The reaction of PEG, activated with either p-nitrophenylchloroformate or cyanuric chloride, with bovine serum albumin

at 4 degrees C reached a plateau within 1 h, whereas protein modification using PEG activated with N,N'-carbonyldiimidazole was rather slow and gave a low yield. The remaining activity of L-asparaginase modified with PNP-PEG was much higher than that of the enzyme modified to the same degree with PEG activated with cyanuric chloride. At a 20 molar excess of PNP-PEG having a molecular weight of 5,000, 55% of the free amino acid groups were modified at 4 degrees C for 2 h, and the modified enzyme still had 33% residual enzyme activity. Immunochemical studies showed that the highly modified enzyme (67% modification with 18% residual enzyme activity) had lost its immunogenicity and had become much less sensitive to protease digestion.

L28 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1995:16065 BIOSIS

DOCUMENT NUMBER: PFEV199598030365
TITLE: Amination of **amino** functions of proteins with
monomethoxypoly (ethylene glycol)-N-succinimide
carbonate.
AUTHOR(S): Nijs, Michelle; Gelbcke, Michel; Azarkan, Mohamed;
Brygier, Jeanne; Guermant, Claude; Baeyens-Volant, Danielle; Musu,
Tony; Paul, Claudine; Looze, Yvan (1)
CORPORATE SOURCE: (1) Protein Chem. Unit, Fac. Med., Univ. Brussels,
Brussels
Belgium
SOURCE: Applied Biochemistry and Biotechnology, (1994) Vol. 49,
No. 1, pp. 75-91.
ISSN: 0273-2289.

DOCUMENT TYPE: Article
LANGUAGE: English

AB Monomethoxypoly(ethylene glycol)-N-succinimide carbonate (SC-**PEG**
) was used to prepare **PEG**-lysozyme, **PEG**-papaya
proteinase 111, **PEG**-catalase, and **PEG**
-lactoperoxidase conjugates. SC-**PEG** produced extensively
modified enzymes under mild conditions (pH 7.0; 25 degree C) within a
couple of hours. **PEG**-enzyme conjugates showed equal or even
greater specific activity provided that low-molecular-
weight substrates were used to evaluate the biological activities.
However, papaya proteinase III and lysozyme lost their proteolytic and
bacteriolytic activities, respectively, on conjugation with **PEG**.
This was most probably because of steric factors, since no drastic
conformational changes could be detected after conjugation of these
enzymes with **PEG** chains. Unlike these enzymes, the secondary
structures of the two hemoproteins were somewhat affected by the
covalent attachment of **PEG** chains as shown by FTIR
experiments. These results confirmed the potential usefulness of SC-
PEG, for which a novel route of synthesis making use of
N,N'-disuccinimidyl carbonate was described.

L28 ANSWER 4 OF 6 MEDLINE

ACCESSION NUMBER: 93289947 MEDLINE
DOCUMENT NUMBER: 93289947 PubMed ID: 8512060
TITLE: Reagents for the preparation of chromophorically labeled
polyethylene glycol-protein conjugates.
AUTHOR: Ladd D L; Snow R A
CORPORATE SOURCE: Medicinal Chemistry Department, Sterling Winthrop
Pharmaceuticals Research Division, Sterling Winthrop Inc.,
Malvern, Pennsylvania 19355.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1993 May 1) 210 (2) 258-61.
Journal code: 4NK; 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 19930723
Entered Medline: 19930709

AB We have developed a new class of reagents (2) for the **covalent**
attachment of **polyethylene glycol** to proteins. These
reagents (2) are the monomethoxypolyethylene glycol esters of
4-fluoro-3-nitrobenzoic acid. The reaction of 2 with lysine epsilon-
amino groups produces a chromophore which can be used to
quantitate the **polyethylene glycol** to protein molar
ratio. Bovine (Zn, Cu) **superoxide dismutase** was used
as a model protein for conjugation with 2. When monomethoxypolyethylene
glycol of average **molecular weight** 2105 was used, a
conjugate was obtained with a **polyethylene glycol** to
protein molar ratio of 8.88 retaining 100% of native enzymatic activity;

monomethoxypolyethylene glycol of average molecular weight 5210 yields a conjugate with a polyethylene glycol to protein molar ratio of 9.96 retaining 73% of native enzymatic activity.

L28 ANSWER 5 OF 6 MEDLINE
ACCESSION NUMBER: 90339014 MEDLINE
DOCUMENT NUMBER: 90339014 PubMed ID: 2166134
TITLE: Spectroscopic characterization of polyethyleneglycol modified **superoxide dismutase**: 1H NMR studies on its Cu₂Co₂ derivative.
AUTHOR: Banci L; Bertini I; Caliceti P; Monsu Scolaro L; Schiavon O; Veronese F M
CORPORATE SOURCE: Department of Chemistry, University of Florence, Italy.
SOURCE: JOURNAL OF INORGANIC BIOCHEMISTRY, (1990 Jun) 39 (2) 149-59.
JOURNAL code: JAR; 7905788. ISSN: 0162-0134.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901012
Last Updated on STN: 19901012
Entered Medline: 19900910

AB Spectroscopic methods have been employed in order to understand the molecular basis of the decrease in enzymatic activity of the antiinflammatory enzyme copper-zinc **superoxide dismutase** (SOD) following the **covalent** binding of polyethyleneglycol (PEG) chains to the protein amino-groups. The PEG modification is a general method recently proposed to improve the therapeutic index of enzymes. 1H NMR spectra on the cobalt substituted PEG-modified SOD, Cu₂Co₂-PEG-SOD, have been recorded. The signals are quite broad with respect to the unmodified enzyme. This has been interpreted on the basis of the effect of **molecular weight** on the linewidth. The analysis has shown that the histidine hydrogens involved in metal binding at the enzyme active site are the same in both native and PEG-modified SOD. Similarly, circular dichroism and absorption spectra indicate that the overall conformation of the metal clusters is not perturbed upon modification. On the other hand, azide titration shows that the affinity constant of N-3 for SOD is largely reduced upon PEG modification (K = 154 M⁻¹ and 75 M⁻¹ for the native and modified SOD, respectively). These results indicate that the decrease in enzymatic activity upon surface modification with PEG is not caused by a perturbation of the active site geometry, but to a decrease in the channeling of the O₂⁻ ion towards the enzyme active site.

L28 ANSWER 6 OF 6 MEDLINE
ACCESSION NUMBER: 77187848 MEDLINE
DOCUMENT NUMBER: 77187848 PubMed ID: 16907
TITLE: Effect of **covalent** attachment of **polyethylene glycol** on immunogenicity and circulating life of bovine liver **catalase**.
AUTHOR: Abuchowski A; McCoy J R; Palczuk N C; van Es T; Davis F F
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1977 Jun 10) 252 (11) 3582-6.
JOURNAL code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197707
ENTRY DATE: Entered STN: 19900314

AB Methoxypolyethylene glycols of 1900 daltons (**PEG-1900**) or 5000 daltons (**PEG-5000**) were covalently attached to bovine liver **catalase** using 2,4,6-trichloro-s-triazine as the coupling agent. Rabbits were immunized by the intravenous and intramuscular routes with **catalase** modified by covalent attachment of **PEG**-1900 to 43% of the **amino** groups (**PEG-1900-catalase**). The intravenous antiserum did not yield detectable antibodies against **PEG-1900-catalase** or native **catalase**, as determined by Ouchterlony and complement fixation methods, whereas the intramuscular antiserum contained antibodies to both **PEG-1900-catalase** and **catalase**. **PEG**-1900 did not react with either antiserum. **Catalase** was prepared in which **PEG-5000** was attached to 40% of the **amino** groups (**PEG-5000-catalase**). This **catalase** preparation did not react with either antiserum. **PEG-1900-catalase** retained 93% of its enzymatic activity; **PEG**-5000-**catalase** retained 95%. **PEG-5000-catalase** resisted digestion by trypsin, chymotrypsin, and a protease from *Streptomyces griseus*. **PEG-1900-catalase** and **PEG-5000-catalase** exhibited enhanced circulating lives in the blood of acatalasemic mice during repetitive intravenous injections. No evidence was seen of an immune response to injections of the modified enzymes. Mice injected repetitively with **PEG-5000-catalase** remained immune competent for unmodified **catalase**, and no evidence of tissue or organ damage was seen.

L28 ANSWER 1 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)

ACCESSION NUMBER: 1998:12669 SCISEARCH

THE GENUINE ARTICLE: BK03M

TITLE: Conjugation of high-molecular weight
poly(ethylene glycol) to cytokines:

Granulocyte-macrophage

colony-stimulating factors as model substrates

AUTHOR: Sherman M R (Reprint); Williams L D; Saifer M G P; French
J A; Kwak L W; Oppenheim J J

CORPORATE SOURCE: MT VIEW PHARMACEUT INC, 871-L IND PK, MENLO PK, CA 94025
(Reprint); NCI, FREDERICK CANC RES & DEV CTR, FREDERICK,
MD 21702

COUNTRY OF AUTHOR: USA

SOURCE: ACS SYMPOSIUM SERIES, (FEB 1997) Vol. 680, pp. 155-169.
Publisher: AMER CHEMICAL SOC, 1155 SIXTEENTH ST NW,
WASHINGTON, DC 20036.
ISSN: 0097-6156.

DOCUMENT TYPE: General (Review), Journal

LANGUAGE: English

REFERENCE COUNT: 71

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The ability of the small receptor-binding protein, recombinant murine
granulocyte-macrophage colony-stimulating factor (GM-CSF), to increase
the

abundance of certain blood cell types in mice was enhanced markedly by
covalent attachment of a single long strand of **PEG**
(30-40 kDa). Potency was not increased further by coupling a second
strand. Such conjugates can be synthesized efficiently by reaction of
protein **amino** groups with **PEG** propionaldehydes in the
presence of NaBH₃CN or with **PEG** p-nitrophenyl carbonates. Both
methods have been used to prepare recombinant human GM-CSF conjugates of
predetermined composition, e.g. **PEG**(1)GM-CSF and **PEG**
(2)GM-CSF, in high yield. These compounds, or analogous derivatives of
other cytokines, purified by ion-exchange and size-exclusion
chromatography, may be suitable candidates for pharmaceutical
development.

09/493,887

L24 ANSWER 5 OF 6 MEDLINE
ACCESSION NUMBER: 90339014 MEDLINE
DOCUMENT NUMBER: 90339014 PubMed ID: 2166134
TITLE: Spectroscopic characterization of polyethyleneglycol
modified **superoxide dismutase**: 1H NMR
studies on its Cu₂Co₂ derivative.
AUTHOR: Banci L; Bertini I; Caliceti P; Monsu Scolaro L; Schiavon
O; Veronese F M
CORPORATE SOURCE: Department of Chemistry, University of Florence, Italy.
SOURCE: JOURNAL OF INORGANIC BIOCHEMISTRY, (1990 Jun) 39 (2)
149-59.
Journal code: JAR; 7905788. ISSN: 0162-0134.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901012
Last Updated on STN: 19901012
Entered Medline: 19900910

AB Spectroscopic methods have been employed in order to understand the
molecular basis of the decrease in enzymatic activity of the
antiinflammatory enzyme copper-zinc **superoxide dismutase**
(SOD) following the **covalent** binding of polyethyleneglycol (**PEG**)
chains to the protein **amino**-groups. The **PEG**
modification is a general method recently proposed to improve the
therapeutic index of enzymes. 1H NMR spectra on the cobalt substituted
PEG-modified SOD, Cu₂Co₂-**PEG**-SOD, have been recorded.
The signals are quite broad with respect to the unmodified enzyme. This
has been interpreted on the basis of the effect of **molecular**
weight on the linewidth. The analysis has shown that the histidine
hydrogens involved in metal binding at the enzyme active site are the
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in both native and **PEG**-modified SOD. Similarly, circular
dichroism and absorption spectra indicate that the overall conformation
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the metal clusters is not perturbed upon modification. On the other hand,
azide titration shows that the affinity constant of N-3 for SOD is
largely
reduced upon **PEG** modification (K = 154 M⁻¹ and 75 M⁻¹ for the
native and modified SOD, respectively). These results indicate that the
decrease in enzymatic activity upon surface modification with **PEG**
is not caused by a perturbation of the active site geometry, but to a
decrease in the channeling of the O₂⁻ ion towards the enzyme active site.

L24 ANSWER 6 OF 6 MEDLINE
ACCESSION NUMBER: 77187848 MEDLINE
DOCUMENT NUMBER: 77187848 PubMed ID: 16907
TITLE: Effect of **covalent** attachment of
polyethylene glycol on immunogenicity and
circulating life of bovine liver **catalase**.
AUTHOR: Abuchowski A; McCoy J R; Palczuk N C; van Es T; Davis F F
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1977 Jun 10) 252 (11)
3582-6.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH:

197707

ENTRY DATE:

Entered STN: 19900314

Last Updated on STN: 19950206

Entered Medline: 19770723

AB Methoxypolyethylene glycols of 1900 daltons (**PEG-1900**) or 5000 daltons (**PEG-5000**) were covalently attached to bovine liver **catalase** using 2,4,6-trichloro-s-triazine as the coupling agent. Rabbits were immunized by the intravenous and intramuscular routes with **catalase** modified by covalent attachment of **PEG**-1900 to 43% of the **amino** groups (**PEG-1900-catalase**). The intravenous antiserum did not yield detectable antibodies against **PEG-1900-catalase** or native **catalase**, as determined by Ouchterlony and complement fixation methods, whereas the intramuscular antiserum contained antibodies to both **PEG-1900-catalase** and **catalase**. **PEG**-1900 did not react with either antiserum. **Catalase** was prepared in which **PEG-5000** was attached to 40% of the **amino** groups (**PEG-5000-catalase**). This **catalase** preparation did not react with either antiserum. **PEG-1900-catalase** retained 93% of its enzymatic activity; **PEG**-5000-**catalase** retained 95%. **PEG-5000-catalase** resisted digestion by trypsin, chymotrypsin, and a protease from *Streptomyces griseus*. **PEG-1900-catalase** and **PEG-5000-catalase** exhibited enhanced circulating lives in the blood of acatalasemic mice during repetitive intravenous injections. No evidence was seen of an immune response to injections of the modified enzymes. Mice injected repetitively with **PEG-5000-catalase** remained immune competent for unmodified **catalase**, and no evidence of tissue or organ damage was seen.